60th Medical Group (AMC), Travis AFB, CA

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

FINAL REPORT SUMMARY

(Please type all information. Use additional pages if necessary.)

PROTOCOL #: FDG20130006A **DATE:** 2 December 2013

PROTOCOL TITLE: "Pilot study of the efficacy of extracellular matrix arterio-venous bypass grafts in a sheep (*Ovis aries*) model".

PRINCIPAL INVESTIGATOR (PI) / TRAINING COORDINATOR (TC): Lt Col Daren Danielson

DEPARTMENT: 60MSGS/SGCH **PHONE #:** 423-2300

INITIAL APPROVAL DATE: 17 January 2013 **LAST TRIENNIAL REVISION DATE:** N/A

FUNDING SOURCE:

1. **RECORD OF ANIMAL USAGE:**

Animal Species:	Total # Approved	# Used this FY	Total # Used to Date
Ovis aries	3	3	3

2. **PROTOCOL TYPE / CHARACTERISTICS:** (Check all applicable terms in **EACH** column)

___ Training: Live Animal	___ Medical Readiness	___ Prolonged Restraint
___ Training: non-Live Animal	___ Health Promotion	___ Multiple Survival Surgery
_X__ Research: Survival (chronic)	___ Prevention	___ Behavioral Study
___ Research: non-Survival (acute)	___ Utilization Mgt.	___ Adjuvant Use
___ Other ( )	__X_ Other (Treatment)	___ Biohazard

3. **PROTOCOL PAIN CATEGORY (USDA):** (Check applicable) ___ C _X_ D ___ E

4. **PROTOCOL STATUS:**

***Request Protocol Closure:**

___ Inactive, protocol never initiated

___ Inactive, protocol initiated but has not/will not be completed

_X_ Completed, all approved procedures/animal uses have been completed

5. **FUNDING STATUS:** Funding allocated: $ $10,080.00 Funds remaining: $ 0.00

6. **PROTOCOL PERSONNEL CHANGES:**

Have there been any personnel/staffing changes (PI/CI/AI/TC/Instructor) since the last IACUC approval of protocol, or annual review? ___ Yes _X_ No

If yes, complete the following sections (Additions/Deletions). For additions, indicate whether or not the IACUC has approved this addition.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
27 DEC 2013	**Final**	**17 Jan 2013 - 27 Dec 2013**

4. TITLE AND SUBTITLE
FDG20130006A "Pilot study of the efficacy of extracellular matrix arterio-venous bypass grafts in a sheep (Ovis aries) model."

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Lt Col Daren Danielson, Maj Lucas P. Neff, Sterling Humphrey, M.D., W. Douglas Boyd, M.D., M.Ed.

5d. PROJECT NUMBER
FDG20130006A

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Clinical Investigation Facility David Grant Medical Center 101 Bodin Circle Travis AFB, CA 94535

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Clinical Investigation Facility David Grant Medical Center 101 Bodin Circle Travis AFB, CA 94535

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Objective: The purpose of this study was to compare early patency and histology of Cormatrix small intestine submucosa arteriovenous fistula grafts in sheep. Methods: Three crossbred sheep were anesthetized, instrumented, and had a 7 cm fistula created between the carotid artery and jugular vein through a midline neck incision. The fistula was created with CorMatrix extracellular matrix. The wounds were closed and the animals recovered. Lovenox was administered starting post-operatively daily for the remainder of the experiment. Duplex ultrasonography was conducted at 1 and 6 weeks, followed by thorough necropsy and histologic evaluation of the fistulas using hematoxylin and eosin and Massons Trichrome stains. Results: Following surgery, two animals had uncomplicated courses without clinical evidence of thrombosis or wound complication. The third animal succumbed from graft failure secondary to a postoperative seroma and wound infection. Duplex examinations revealed patent fistulas with normal vessel diameters, flow velocities, and spectral patterns. Upon post mortem, there was a lack of perivascular inflammation and tissue reaction. Histologic assessment confirmed patency without evidence of thrombosis or inflammatory infiltration. ECM was well populated with cells and near complete luminal endothelial cell coverage was present by four weeks. Conclusion: In this pilot study, the Cormatrix extracellular matrix performed well in a sheep A-V fistula graft model.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**3**	

ADDITIONS: (Include Name, Protocol function - PI/CI/AI/TC/Instructor, IACUC approval - Yes/No)

_____

_____

DELETIONS: (Include Name, Protocol function - PI/CI/AI/TC/Instructor, Effective date of deletion)

_____

_____

7. PROBLEMS / ADVERSE EVENTS: Identify any problems or adverse events that have affected study progress. Itemize adverse events that have led to unanticipated animal illness, distress, injury, or death; and indicate whether or not these events were reported to the IACUC.

One of the three sheep had a failure of the graft anastomosis, followed by fatal hemorrhage. The graft failed after a seroma with wound infection developed at the surgical site.

8. REDUCTION, REFINEMENT, OR REPLACEMENT OF ANIMAL USE:

REPLACEMENT (ALTERNATIVES): Since the last IACUC approval, have alternatives to animal use become available that could be substituted in this protocol without adversely affecting study or training objectives?

No.

REFINEMENT: Since the last IACUC approval, have any study refinements been implemented to reduce the degree of pain or distress experienced by study animals, or have animals of lower phylogenetic status or sentience been identified as potential study/training models in this protocol?

Yes. After the bad outcome with the first sheep, the AV recommended that cyanoacrylate adhesive be used to seal the skin wound after staples had been placed. A sterile dressing was then used to protect the wound for 3 – 4 days.

REDUCTION: Since the last IACUC approval, have any methods been identified to reduce the number of live animals used in this protocol?

No. This study used a pilot approach to demonstrate that it was possible to create an A-V fistula using extracellular matrix.

9. PUBLICATIONS / PRESENTATIONS: (List any scientific publications and/or presentations that have resulted from this protocol. Include pending/scheduled publications or presentations).

None.

10. Were the protocol objectives met, and how will the outcome or training benefit the DoD/USAF?

Yes. Valuable experience in using extracellular matrix for A-V fistula formation was gained. If shown successful in a future study, this procedure may provide an advanced therapeutic option for military vascular surgeons.

11. PROTOCOL OUTCOME SUMMARY: (Please provide, in "ABSTRACT" format, a summary of the protocol objectives, materials and methods, results - include tables/figures, and conclusions/applications.)

Objective: The purpose of this study was to compare early patency and histology of Cormatrix™ small intestine submucosa arteriovenous fistula grafts in sheep.
Methods: Three crossbred sheep were anesthetized, instrumented, and had a 7 cm fistula created between the carotid artery and jugular vein through a midline neck incision. The fistula was created with CorMatrix™ extracellular matrix. The wounds were closed and the animals recovered. Lovenox was administered starting post-operatively daily for the remainder of the experiment. Duplex ultrasonography was conducted at 1 and 6 weeks, followed by thorough necropsy and histologic evaluation of the fistulas using hematoxylin and eosin and Masson's Trichrome stains.
Results: Following surgery, two animals had uncomplicated courses without clinical evidence of thrombosis or wound complication. The third animal succumbed from graft failure secondary to a postoperative seroma and wound infection. Duplex examinations revealed patent fistulas with normal vessel diameters, flow velocities, and spectral patterns. Upon post mortem, there was a lack of perivascular inflammation and tissue reaction. Histologic

assessment confirmed patency without evidence of thrombosis or inflammatory infiltration. ECM was well populated with cells and near complete luminal endothelial cell coverage was present by four weeks.
Conclusion: In this pilot study, the Cormatrix extracellular matrix performed well in a sheep A-V fistula graft model.

(PI / TC Signature)

18 Dec 13
(Date)